Sugar Production from Bioenergy Sorghum by Using Pilot Scale Continuous Hydrothermal Pretreatment Combined with Disk Refining

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Abstract

Chemical-free pretreatments are attracting increased interest because they generate less inhibitor in hydrolysates. In this study, pilot-scaled continuous hydrothermal (PCH) pretreatment followed by disk refining was evaluated and compared to laboratory-scale batch hot water (LHW) pretreatment. Bioenergy sorghum bagasse (BSB) was pretreated at 160 to 190°C for 10 minutes with and without subsequent disk milling. Hydrothermal pretreatment and disk milling synergistically improved glucose and xylose release by 10 to 20% compared to hydrothermal pretreatment alone. Maximum yields of glucose and xylose of 82.55% and 70.78%, respectively were achieved, when BSB was pretreated at 190°C and 180°C followed by disk milling. LHW pretreated BSB had 5 to 15% higher sugar yields compared to PCH for all pretreatment conditions. The surface area improvement was also performed. PCH pretreatment combined with disk milling increased BSB surface area by 31.80 to 106.93%, which was greater than observed using LHW pretreatment.

Keywords: Bioenergy sorghum, Sugar production, Pilot-scale continuous hydrothermal pretreatment, Disk milling, Surface area

1. Introduction

There is considerable interest in developing sugar production from lignocellulose as a feedstock for production of sustainable fuels and chemicals. The major barriers are developing dependable supply chains and lowering sugar processing costs. Overcoming the recalcitrant lignocellulosic biomass structure is the major barrier for low-cost biomass processing (Chen, et al., 2014). Hence, efficient deconstruction of lignocellulosic biomass into mono/soluble sugars is critical for its future (Himmel, et al., 2007, Balch, et al., 2017).

Developing cost-effective and feasible pretreatment technologies, which fractionate cellulose, hemicellulose, and lignin and minimize the formation of sugar degradation products have been the major goals of lignocellulosic feedstock refinery (Meng et al., 2015). The major approaches to reducing biomass recalcitrance include chemical and physical approaches (Menon & Rao, 2012). Among various pretreatment technologies, dilute acid pretreatment has been the most studied and furthest developed at commercial scales (Elander, et al., 2005, Yang & Wyman, 2008). However, dilute acid pretreatment has several disadvantages including additional neutralization and detoxification processes are required after the pretreatment, high capital costs for constructing corrosion resistant reactors, higher chemical costs, and the formation of side-products from xylose degradation that inhibit enzymatic hydrolysis and fermentation (Yang & Wyman, 2008, Kim et al., 2011).

Hot water or hydrothermal pretreatment is an eco-friendly alternative to dilute-acid. The kinetic mechanism is similar because it depends upon hydronium ions formed at greater concentrations at high temperatures and pressures. The hydronium ions catalyze the hydrolysis of hemicellulose into soluble oligo- and monosaccharides. Moreover, because most of the hydrolyzed xylan is in the form of oligomers and not monosaccharides, degradation of xylose to

70 furfural is limited (Mosier et al., 2005, Mosier, 2013, Jönsson & Martín, 2016). However,
71 higher pretreatment temperature (20 to 50ºC) and longer processing time (5 to 10 min) are
72 required than for dilute acid pretreatment because of the less acidic solution to obtain the optimal
73 sugar yields (Mosier, 2013). Furthermore, there is no need to add neutralization or detoxication
74 steps after the chemical free hydrothermal pretreatment which can reduce the total capital
75 investment and operation costs. An alternative to increasing pretreatment severity is to introduce
76 a post-pretreatment mechanical refining step (Lin, et al., 2010, Chen, et al., 2013, Chen, et al.,
77 2014, Kim et al, 2016, Wanget al., 2018, 2019).

78 Mechanical refining technologies are used by the paper and pulp industry for cutting,
79 shearing, and compression to reduce biomass particle size, decrease cellulose crystallinity and
80 increase specific surface area (Nakagaito & Yano, 2004, Barakat et al., 2013, Gharehkhani, et al.,
81 2015). Ball milling, disk milling, extrusion, PFI (Papirindustriens Forskningsinstitutt) milling,
82 and Szego milling are commonly used for mechanical refining (Kim et al., 2016[b]). High energy
83 consumption is the major disadvantage of mechanical milling. The mechanical milling following
84 hydrothermal/chemical pretreatment reduces energy consumption by up to 95% because
85 hydrothermal/chemical pretreatment softens the cell wall fibers (Lee et al., 2010, Zhu et al.,
86 2010). Additionally, mechanical refining, by either disk or Szego milling, after
87 hydrothermal/chemical pretreatment is positively synergistic with improved sugar yields of up to
88 9.45-fold and 2.03-fold compared to solely milling or hydrothermal/chemical pretreatment,,
89 respectively (Kim et al., 2016[b]). Thus, high sugar yields reduce total sugar production cost. For
90 pilot and industrial scale, disk milling is favored among these mechanical refining technologies
91 due to its economy and scalability (Barakat et al., 2013). The minimum sugar selling price from
92 a similar process was estimated from 0.42 to 0.47 $/kg by Chen et al., (2015).

Sorghum is a productive and drought tolerant species and widely grown for cereal, sugar, feeds, and ethanol production in the world. Unlike grain and sweet sorghums, the structural carbohydrate of bioenergy sorghum provides sources for biofuels production. The abundant biomass yields of bioenergy sorghum, in excess of 80 Mg ha^{-1} (fresh weight) and 20 Mg ha^{-1} (dry weight), favor the lignocellulosic applications in biofuel and biochemical developments (McBee et al., 1987, Rooney et al., 2007). Additionally, bioenergy sorghum is regarded as a dedicated bioenergy feedstock in several different schemes. For the aspect of crop residue and bagasse, it is readily available for cellulosic conversion after harvest. As to sorghum growing cost, great level of drought tolerance and wide adaptation to the environment make the costs associated with sorghum lower than other crops (Rooney et al., 2007). These advantages allow more flexible and sustainable management for bioenergy sorghum in further applications.

For industrial operations, the recently introduced industrial-scale integrated continuous hydrothermal pretreatment (D3MAX process) is used for cellulosic ethanol production from corn fiber and residues derived from corn dry-grind process (D3MAX, 2018, ACE Ethanol, 2018). This process maximizes the ethanol yields and profits for corn ethanol plants. However, this technology has not been used in lignocellulose biomass refinery. The heart of the process is a continuous flow high-solids reactor, which is suitable for hot water pretreatment. In this study, this process was combined with mechanical disk refining to evaluate our process at pilot-scale for sugar production. To determine the effect of scale-up at the microscopic scale, fibers pretreated in a commonly used laboratory batch system and the pilot system were compared for surface area.

2. Materials and Methods

2.1 Feedstock

Bioenergy sorghum (TAM17800) was harvested in September 2018 from experimental field plots in Urbana, IL. The bioenergy sorghum was shredded while field harvesting. After harvest, biomass was ambiently dried until the moisture content was less than 10%. Dried biomass was ground using a hammer mill (W-8-H, Schutte-Buffalo Hammermill, Buffalo, NY) equipped with a 3 mm sieve size. The ground biomass was stored in sealed containers at 4°C.

2.2 Pilot-scale continuous hydrothermal pretreatment (PCH)

A pilot scaled continuous pretreatment reactor (SüPR•2G Reactors, AdvanceBio system LLC., Milford, OH) was used for hydrothermal treatment. It consists of a 0.11 m^3 feed hopper with 89 mm diameter open flight and full pitch equalizing screw feeder, a reactor designed for 20.7 barg (300 psig) at 204°C with an 150 mm diameter and 1.37 meters long screw, discharge system with two full port ball valves, a flash tank with 250 mm diameter and 750 mm straight side, a condenser, and a receiving tank (Fig.1).

The reactor temperature is controlled by setting the steam pressure corresponding to the desired temperature using the pressure controller, and the steam is directed to the inlet nozzle in the reactor. The screw feeder receives feedstock from the feed hopper and discharges into the reactor. The compression in the screw aids in the formation of the plug at the entrance of the pressure zone to seal between atmospheric pressure and the high pressure of the reaction chamber. The pretreatment time is controlled by setting the feeding/flow rate of the screw feeder within the reactor. After the reaction, the feedstock is transported by the discharge system. The two full port ball valves open and close in alternative manner with air-to-open and air-to-close actuators to discharge pretreated biomass into the flash tank before entering the receiving tank.

Before the pretreatment, the moisture content of bioenergy sorghum was adjusted to 50%. Four pretreatment temperatures conditions were chosen (160, 170, 180, and 190 °C) and a holding time of 10 min. The severity parameter (R_o) of pretreatment was defined by the following equation (Eq. 1), where t is reaction time (min), T is pretreatment temperature (°C), and T_R is reference temperature (100 °C). The logarithm severity factor is represented by Log R_o (Overend et al., 1987, Kim et al., 2016, Wang et al., 2018).

$$R_o = t \times exp[(T - T_R)/14.75] \qquad Eq.1$$

2.3 Lab-scale hot water pretreatment (LHW)

A fluidized sand bath (IFB-51 Industrial Fluidized Bath, Techne Inc., Burlington, NJ) was used for the lab scaled hot water pretreatment experiments. Bioenergy sorghum was loaded in 50 ml stainless steel pipe reactors (316 stainless with 10.478 cm length × 1.905 cm outer diameter × 0.165 cm wall thickness tubing, SS-T12-S-065-20, Swagelok, Chicago Fluid system Technologies, Chicago, IL). The reactors were capped with 316 stainless steel caps (SS-1210-C, Swagelok, Chicago Fluid system Technologies, Chicago, IL).

LHW was performed at 10% w/w solid loading with reaction temperatures of 160, 170, 180, or 190°C and 10 min holding time. The *in situ* reaction temperature was monitored using a thermocouple (Penetration/Immersion Thermocouple Probe Mini Conn (-418 to 1652°F), Mc Master-Carr, Robbinsville, NJ) inserted into one reactor and connected to a data logger (HH306/306A, Datalogger Thermometer, Omega, Stamford, CT). Each pretreatment was heated rapidly to the target temperature in the sand bath (within 5 min). After the pretreatment, reactors were transferred to a water bath to quench the reaction. The severity factor was as well calculated for LHW.

159 *2.4 Disk milling/refining*

160 Pretreated bioenergy sorghum from PCH and LHW were disk milled "as is" without
161 separation, washing, and drying. An electrical power disk mill (model 4E, Quaker City grinding
162 milling, Straub Co., Philadelphia, PA) with an output speed of 89 rpm was used. The disk mill
163 consists of a stationary and a rotating disks. The distance between two disks was set at the
164 minimum gap, and the samples were milled sequentially three times (Kim et al., 2016).

165 *2.5 Compositional analysis of raw and pretreated bioenergy sorghum*

166 Raw and pretreated sorghum samples were freeze dried (Laconco, Kansas City, MO) for
167 72 hr. The chemical composition of biomass was analyzed following the Laboratory Analytical
168 Procedure for biomass analysis from National Renewable Energy Laboratory (NREL).

169 Extractives were removed by deionized water and ethanol extraction using Soxhlet
170 method based on NREL/TP-510-42619 (2008). Two-step acid hydrolysis was adapted for
171 carbohydrate contents in biomass (NREL/TP-510-42618, 2012). After acid hydrolysis,
172 hydrolyzed samples were vacuum filtered using filter crucibles. The filtrates were analyzed for
173 carbohydrate concentration by HPLC; acid soluble lignin (ASL) was measured by
174 spectrophotometer. The solids remained in filter crucibles were analyzed for acid insoluble lignin
175 (AIL) and ash contents by first drying in a static oven and next ashing in a muffle oven
176 (NREL/TP-510-42622, 2008).

177 *2.6 Enzymatic hydrolysis*

178 Enzymatic hydrolysis was performed after pretreatment and disk milling to measure
179 sugar recovery from the pretreated biomass following standard protocol NREL/TP-5100-63351
180 (2008). Pretreated or disk milled samples were transferred into sterilized corning tubes at 10%

(w/w) solids loading. The pH was adjusted by adding 1 M sodium citrate buffer (pH 4.5) to a final concentration of 0.05 M and pH of 5.0. Finally, the cellulase and hemicellulase cocktails and deionized water were added to bring to 10% solids content. Cellic® Ctec2 (Novozymes North America, Inc., Franklinton, NC, USA) at 16.95 mg cellulase protein/g dry substrate and NS 22204 (Novozymes North America, Inc., Franklinton, NC, USA) at 4.24 mg cellulase protein/g dry substrate were added. The hydrolysis process was performed at 50 °C with 120 rpm constant shaking for 72 hr using a shaker/incubator. For each pretreated biomass, enzymatic hydrolysis was performed in triplicate, and monosaccharides were measured by HPLC. Additionally, enzyme blanks were prepared and used to correct for background sugars. The sugar recovery was defined as the ratio of sugar yield to theoretical yield from carbohydrate contents in raw bioenergy sorghum samples.

2.7 HPLC analysis for sugars and inhibitors

Liquid samples from chemical compositional analyses (two-step acid hydrolysis) and hydrolysates from enzymatic hydrolysis were collected, centrifuged at 9000×g, and the supernatants were filtered through 0.2 μm PTFE filters before HPLC analysis. HPLC (Bio-Rad Aminex HPX-87H, Biorad, Hercules, CA) was used to determine concentrations of monosaccharides, organic acid (acetate), and other inhibitors (furans).

2.8 Surface area analysis

Freeze dried raw and pretreated biomass was used for surface area analysis following Langmuir adsorption (Wiman, et al., 2012). Langmuir adsorption was performed with 1% freeze dried biomass in 0.03 M phosphate buffer (pH 6) with 1.4 mM sodium chloride and incubated at 60 °C with 180 rpm constant shaking for 24 hr. DR28 (Sigma Aldrich) was used as the dye. A

series of increasing DR28 concentration from 0.00 to 6.00 was analyzed for each bioenergy sorghum sample. After incubation, the supernatants were obtained by centrifuging for 5 min at 740×g. The absorbance of each supernatant sample and reference solution was measured by spectrophotometer at 498 nm to calculate by difference the amounts of adsorbed dye.

The maximum adsorption capacity was determined by assuming that DR28 adsorbs as dimer aggregates under experimental conditions (Inglesby & Zeronian, 2002) and using non-linear regression (Matlab,Mathworks, Natick, USA). Furthermore, the occupied cellulose area by one aggregate was assumed to constitute 30% of the dimer Connolly surface area of 813 $Å^2$. Therefore, 1g of absorbed dye represents 1055 m^2 of surface (Inglesby & Zeronian, 2002, Inglesby et al., 2002).

2.9 Statistical analysis

Chemical composition of biomass samples and sugar yields were calculated on biomass dry basis. Analyses of variance (ANOVA) and Tukey HSD tests were performed using R (V.3.5.2) to investigate the significance with a p value of 5% ($p<0.05$).

3. Results and discussion

3.1 Effect of pretreatments on bioenergy sorghum composition

3.1.1 Compositional analysis of raw and pretreated biomass

Bioenergy sorghum and pretreated solids were analyzed for composition using the standard NREL fiber method. The compositions of raw (untreated) and pretreated bioenergy sorghum TAM17800 on a total dry mass basis are listed in Table 1. The measured components accounted for 91.6 – 97.2% of the total biomass for the samples.

225 Bioenergy sorghum contained 60.73% carbohydrates (glucan and xylan) and the
226 remaining components (in order of abundance) were acid insoluble lignin (AIL), extractives, ash,
227 and acid soluble lignin (ASL). The soluble (free) sugar concentrations (i.e. glucose, fructose, and
228 sucrose) were below the detection limit of our HPLC. This was not unexpected because the crop
229 has been allowed to form grain heads. The water/ethanol extractives increased with pretreatment
230 temperature (severity factor). This was especially evident for samples treated at 190°C, where it
231 increased to 28.13% and 27.28% for PCH and LHW, respectively. This trend is similar to what
232 Wang et al (2018) observed for sugarcane bagasse and much of this can be accounted for by
233 hydrolysis of xylan and release of lignin at high pretreatment temperature. These hydrolyzed
234 xylan and released lignin were obtained by water/ethanol extraction. Lignin (AIL and ASL) was
235 invariant with pretreatment except at the highest severity. Cellulose and ash contents were
236 invariant with all pretreatment conditions. Ash contents were slightly higher for the PCH
237 samples (4.39%) than for either the raw (2.72%) or LHW samples (2.17%) which may relate to
238 solids loading in the pretreatment. Ash was solubilized along with lignin and xylan in the black
239 liquor at the high pretreatment severity in lower solids loading (10%, w/w) LHW pretreatments;
240 however, ash remained with solids in PCH pretreatments.

241 The hydrothermal pretreatment at high temperature (e.g. large severity factor) releases
242 large amounts of xylan and lignin (Mosier et al., 2005, Pérez, et al., 2008, Hashmi, et al., 2017).
243 When the pretreatment temperature increased to 190°C for PCH and LHW, 52.76% and 64.48%
244 of xylan were solubilized, respectively. In the case of lignin (the sum of AIL and ASL), as the
245 pretreatment severity factor increased from 2.77 to 3.65, 14.92% and 19.44% of the lignin was
246 removed by PCH and LHW, respectively. That lignin remained with the solids at lower severities
247 does not preclude melting and flow of the lignin away from the fiber bundles. Besides scale, the

PCH and LHW reactions vary in solids loading. The lower solids loading (10%, w/w) used for LHW allows for a higher hydronium ion concentration during the reaction, which facilitates greater lignin removal and xylan solubilization than for PCH. A larger concentration of water will also affect solute and ion concentration gradients and solubility limits, which might be especially relevant for lignin containing compounds because it is hydrophobic. Xylan solubilization and lignin removal (away from the cellulose fibers) are considered necessary to ensure cellulose accessibility for enzymatic hydrolysis; therefore, they are considered as critical indicators of pretreatment efficiency (Leu & Zhu, 2013).

Chemical composition changed slightly under mild pretreatment conditions (S.F. 2.77 to 3.36), but xylan solubilization and lignin removal increased dramatically when the severity factor increased to 3.65 for both PCH and LHW. Similar results were for sugarcane bagasse (Wang et al., 2018), where lignin removal and xylan solubilization when treated at 160 and 200°C with hot water increased from 10.86 to 45.62% and 1.92 to 81.50%, respectively.

3.1.2 Inhibitor generation from hydrothermal pretreatments

Furfural and acetic are the two major inhibitors generated by hot water/hydrothermal processing (Jönsson & Martín, 2016). Acetic acid arises from hydrolysis of acetyl groups that decorate hemicellulose sidechains and furfural from dehydration of pentoses. Concentrations of inhibitors formed during the pretreatment are listed in Table 2. Results are not available for inhibitors formed during the PCH pretreatments because exiting material had moisture contents of 60 to 65% and, therefore, did not contain free water. Thus, Table 2 only presents inhibitor results for LHW.

From the results, no levulinic acid and 5-hydroxymethylfurfural (HMF) were detected after LHW indicating the degradation of glucose derived from cellulose was negligible. This result infers that the cellulose remained intact during LHW pretreatments, which is desirable. Other inhibitors, including formic acid, acetic acid, lactic acid, and furfural, were generated at the maximum severity (e.g. 190°C). In LHW pretreatment with a 3.65 severity factor, there were 15.93 mg, 4.14 mg, 4.5 mg, and 2.2 mg of acetic acid, furfural, formic acid, and lactic acid formed per 1 g (db) of bioenergy sorghum, respectively. As furfural and formic acid arise from xylose dehydration, reaction severity should be chosen to balance xylan hydrolysis and xylose degradation. Wang et al., (2018) observed for LWH that furfural and formic acid were first observed when the temperature was set over 180°C, and dramatically increased at 200°C. Additionally, the lactic acid, a potential inhibitor for yeast growth and metabolism in further fermentation, were generated by lactic acid bacteria contamination (Narendranath et al., 2001)

According to literature, *S. cerevisiae*, commonly used for ethanol fermentation, is inhibited by 6 g/L of furfural, 0.5-4 g/L of acetic acid, and 4% w/v of lactic acid (Banerjee et al., 1981, Larsson, et al., 1999, Graves et al., 2006). These inhibitor concentrations are far above the concentrations observed even at 190°C.

3.2 Effect of hydrothermal pretreatment combined with disk milling on sugar yields

3.2.1 Sugar yields from PCH pretreatment

Hydrothermal/hot water pretreatment and disk refining were paired to maintain optimal sugar yields under reduced severity/energy input conditions (Kim et al, 2016). Pretreatment temperature and disk refining process are critical factors increasing sugar yields from unpretreated biomass ($p < 0.05$). Glucose and xylose yields from PCH are graphed in Fig. 2. Pretreatment temperature is a critical factor in determining enzymatic sugar yields as evidenced

292 by it appearing as an exponential in the severity equation. Single-stage (only hydrothermal)
293 pretreatment at 160°C did not improve sugar yields compared to raw (untreated) sorghum
294 sample. When the pretreatment temperature was increased from 160ºC to 180°C, the glucose
295 and xylose yields increased 1.3 and 1.8 fold compared to untreated biomass, respectively. At
296 190°C, PCH gave the highest glucose (74.70%) and xylose (68.80%) yields.

297 Disk milling the pretreated biomass is expected to disrupt the plant cell matrix and
298 defribillated the cellulose fiber bundles and as a result increases accessibility of cellulose fiber to
299 cellulases and improves sugar yields. Adding a disk milling step following hydrothermal
300 pretreatment, improved sugar yields 10-20% compared to the single-stage pretreatment results.
301 Specifically, glucose yields were improved or similar at all pretreatment temperatures: 26.5%
302 (160°C), 21.4%, (170°C), 33.4% (180°C), and 10.5% (190°C, not significant). Xylose yields
303 were improved by disk milling at all temperatures: 36.0% (160°C), 21.2% (170°C), and 23.4%
304 (180°C) except 190°C (-0.2%, not significant). Presumably at the highest severity 3.65, gains in
305 xylan hydrolysis were canceled out by increases in xylose deconstruction.

306 Therefore, following hydrothermal pretreatment with disk milling allowed for a reduction
307 of 10°C in reaction temperature without compromising sugar yield. For examples, pretreating at
308 paired temperatures with and without disk refining gave: 170°C (55.7%) versus 180°C (58.1%);
309 180°C (77.5%) versus 190°C (82.6%). A similar trend was observed for xylose yields at 180°C
310 with disk milling (70.8%) and 190°C without (68.8%).

311 *3.2.2 Sugar yields from LHW pretreatment*

312 Next, we were interested to see if a laboratory scaled system can be used to predict
313 results for the PCH based upon severity factors. This would allow testing and semi-optimizing

pretreatment conditions on the laboratory scale for new sources of biomass saving time and resources. That they would have similar kinetics is not assured because the PCH operates with continuous flow and at very high solids. In contrast, LHW occurs as a static reaction and, therefore, is operated at 10% solids to promote uniform heat transfer. The sugar yields from the LHW experiments are presented in Fig. 3.

Similar reaction patterns with temperature were observed for PCH and LHW pretreatments. For LHW, pretreatment temperature and disk refining process are the critical factors ($p < 0.05$) in improving the sugar recovery. Disk milling also improved glucose yields at all reaction temperatures: 160°C (24.9%), 170°C (18.1%), 180°C (30.6%), and 190°C (8.48%). Xylose yields were likewise improved by 19.9%, 13.0%, and 14.8% at 160°C, 170°C, and 180°C, respectively. However, at 190°C, the xylose yields decreased because of increased degradation (Table 2). Maximum glucose yields (e.g. 98.5%) were realized at 180°C with disk milling at 190°C for both conditions. Additionally, the optimal xylose yield was obtained at LHW set at 180°C combined with disk milling.

Furthermore, the sugar yields increased dramatically when the reaction severity increased to 3.36. This result indicated that 180°C was the appropriate reaction temperature for LHW with disk milling based upon the amount of sugars released (>80%) with minimal xylose degradation (see below). Wang et al., (2018) likewise observed that the glucose yields achieved over 85% when the reaction temperature was set to 180°C and combined with three cycles of disk milling.

3.2.3 Inhibitors concentration in hydrolysates

Inhibitors generation from the enzymatic hydrolysis is critical for further applications, such as fermentation, and other chemical conversions and syntheses. The inhibitor

concentrations in the hydrolysates from PCH and LHW pretreatments combined with disk milling process following enzymatic saccharification are presented in Table 3. (Table 2 lists values for the pretreatment liquor prior to hydrolysate.)

None of the hydrolysates contained detectable concentrations of either HMF or furfural. This is predictable for LWH based upon the inhibitor profile from hydrolysate liquor (Table 2) and for both pretreatments agrees with cellulose being retained (Table 1). However, a verily detectable concentration of levulinic acid was detected for the highest PCH severity pretreatment (190°C). There were modest amounts of acetic acid and formic acid released, which are associated with sugar degradation reactions. As expects, these concentrations rise with severity. More acetic and formic acids were detected for the LHW than for the PCH. This likely reflects evaporation during flashing and possibly increased xylan hydrolysis for the LHW pretreatment. Additionally, the lactic acid was observed from both PCH and LHW pretreatments. However, its concentrations in hydrolysates from PCH and LHW pretreatments were far below the concentration (4% w/v), which would inhibit the further fermentation (Graves et al., 2006).

It is notable that disk refining allows for the pretreatment temperature to be lowered from 190°C to 180ºC without a loss in sugar yields. Operating at this lower temperature is favorable in terms of reducing inhibitor concentrations.

3.3 Change in surface areas following hydrothermal pretreatment and disk milling

Pretreatments are optimized to break down recalcitrant plant cell wall structures. Mechanical refining disrupts the plant cell wall matrix and reduces biomass particle sizes, thereby, exposing cellulose fibers for ceullase hydrolysis. Improvement of surface area is predictive of hydrolysis efficiency (Kleine et al., 2013, Pihlajaniemi, et al., 2016). Surface areas

358 for raw biomass sorghum and all pretreated samples are graphed on Fig. 4. All the pretreatments
359 exposed more surface area compared to the raw biomass and the areas increased with
360 pretreatment severity.

361 PCH was observed to improve surface area by 31.80-106.93% compared to the raw
362 bioenergy sorghum (86.93 m^2). The pretreatment temperature and disk refining were critical in
363 improving external surface area ($p < 0.05$). The surface area increased to 114.57 m^2, 129.08 m^2,
364 131.82 m^2, and 143.27 m^2 at 160°C, 170°C, 180°C, and 190°C, respectively. For PCH
365 pretreatment, the rapid pressure release upon exiting the reactor reduced biomass particle size
366 and likely loosened the biomass cell wall structure. After the disk milling process, the surface
367 area (179.88 m^2) was over one fold higher than the raw bioenergy sorghum at 190°C
368 pretreatment. Additionally, disk milling improved surface area by 3.27% to 25.55% when
369 pretreatment temperature increased from 160°C to 190°C. Disk milling is thought to improve
370 cellulose accessibility by breaking microfibril cross-links (Zhu, 2011).

371

372 For LHW pretreatments, the increased pretreatment temperatures resulted in greater
373 surface area; however, the improvement was not as extensive as observed for PCH
374 pretreatments. Surface area increased especially when the pretreating at 180°C and 190°C and
375 the maximum value was 111.09 m^2, for biomass pretreated at 190°C and disk refined. The disk
376 milling slightly increased the surface area by 1.50-5.50% from the single-stage LHW
377 pretreatments; however, it was not a critical factor ($p = 0.24$) in increasing the surface area.
378 Comparing LHW and PCH surface areas, it is evident that PCH samples had much larger surface
379 areas and disk refining had a more pronounced effect on PCH samples than LWH, albeit for
380 180ºC and 190ºC pretreatments. One possible explanation is that the steam explosion step in the
381 PCH – the LWH samples were cooled by quenching the sealed tubes in a water bath – caused the
382 biomass particles to fragment and weakened the cell wall matrix. This is something will require
383 further study to better understand. However, it is promising that scaling had a beneficial effect on
384 changing surface area.

385 *3.4 Comparison between PCH and LHW*

386 An important conclusion of this study is that it is feasible to effectively pretreat
387 herbaceous biomass with just water using a pilot scale continuous flow reactor, which is also
388 marketed for industrial scale. However, it is expensive to optimize new sources of biomass at the
389 pilot scale and often infeasible for experimental varieties produced in limited quantities.
390 Therefore, parallel experiments were conducted using a popular pretreatment assembly that
391 consists of screw sealed tube reactors heated at low solids (e.g. 10%w/w) in a fluidized heating
392 bath.

393 In PCH pretreatments, the optimal glucose (82.55% of max) and xylose (70.78%) yields
394 were obtained from the pretreatment at 190°C and 180°C combined with disk milling. For LHW
395 pretreatments, the reaction at 180°C combined with disk milling resulted in the maximum
396 glucose (98.47%) and xylose (66.83%) yields. For both pretreatments, disk refining elevated
397 glucose yields by 10-20%. Though the sugar yields from PCH pretreatments were lower than
398 that from the LHW pretreatments, the PCH pretreatment at the optimal condition (190°C
399 combined with three disk milling cycles) had a similar or higher glucose recovery than previous
400 studies. Kim et al., (2016) reported 79% glucose yield from corn stover treated by hot water
401 pretreatment combined with 9 cycles of disk milling. The 95.8% of glucose yields were reported
402 by Wang et al., (2018) by using hot water pretreatment with 3 disk milling cycles for sugarcane
403 bagasse. Chen et al., (2014) reported 85% glucose recovery from pilot scale alkaline
404 deacetylation combined with 9 cycles of disk milling.

405 The glucose yields from PCH pretreatments achieved 80-90% of that from the LHW
406 pretreatments. From the results shown in Table 2 and Table 3, for LHW, furfural was generated
407 at a reaction temperature of 190°C, and the concentrations of acetic and formic acids in the
408 hydrolysates were higher than for the hydrolysates from PCH pretreatments. Perhaps the high
409 water loading in the LHW pretreatments resulted in great amounts of hydronium ions generated
410 per gram of biomass, especially at the high reaction temperatures. These results indicated the
411 LHW pretreatments had better effects on removing/solubilizing xylan than the PCH
412 pretreatments. For PCH, the biomass was heated with direct steam injection and cooled by
413 explosive flashing upon exiting the reactor. Also, because the PCH includes a much more
414 efficient heat transfer/mixing regime, the biomass is able to be treated at 40-60% w/w solid.
415 Higher solids can introduce mixing inefficiencies, lowers solvent effects, and reduces availability

of hydronium ions per biomass. Certainly the 10% w/w solids loading used for the LHW is infeasible. Given differences in scale and solids loading, it is very promising that scaling up still allowed for reasonably high glucose and xylose yields. One area for future improvement might be to further optimize the refining step.

For surface area improvements, the PCH pretreatments had better effects than the LHW pretreatments. The disk milling process also increased the surface area, especially for the PCH pretreatments. The surface area improvement increased when the PCH reaction severity increased. These results were related to the characteristics of the pretreatments. In PCH pretreatments, the sudden pressure release (steam explosion) after the reaction reduced the biomass particle size and loosened the plant cell wall structure. Hence, it made disk refining more effective at increasing biomass surface area. However, the larger surface area did not lead to higher sugar release. The surface area improvement is a feature related to the efficiency of pretreatment, size reduction, and fiber exposure. Moreover, it is a predictor and not a measure of enzymatic hydrolysis. The sugar yields from enzymatic hydrolysis is related to enzyme digestibility and accessibility, which includes protein molecular size (Srisodsuk et al., 1993), diffusion of enzyme to a pore (Bubner et al., 2012), biomass pore size and distribution (Karimi & Taherzadeh, 2016), biomass internal surface (Shafiei et al., 2015), etc. The penetration of enzyme through plant cell wall and the binding between enzyme and cellulose are critical factors in increasing enzyme performance (Yang et al., 2011). Consequently, accessible surface area is not the only factor affecting the efficiency of enzymatic hydrolysis (Khodaverdi et al., 2012). Most notably, however, results for the LHW and PCH were similar enough to give assurance that LHW can be used for early-stage validation and optimization.

4 Conclusions

The industrially based pilot-scale continuous hydrothermal (PCH) pretreatment and disk refining was successfully scaled up from 40 ml reactions using pipe reactors and fluidized sand bath and disk refiner. The optimal glucose yield of 82.55% and xylose yield of 70.78% were obtained from the pretreatment severity of 3.36 based on beginning carbohydrate contents. Additionally, no furans were detected in the enzymatic hydrolysate. Moreover, PCH was much more effective at increasing surface area than the LHW due to the inclusion of a steam explosion step. In conclusion, the chemical-free PCH and disk refining shows promise for high conversion yields.

(E-supplementary data for this work can be found in e-version of this paper online)

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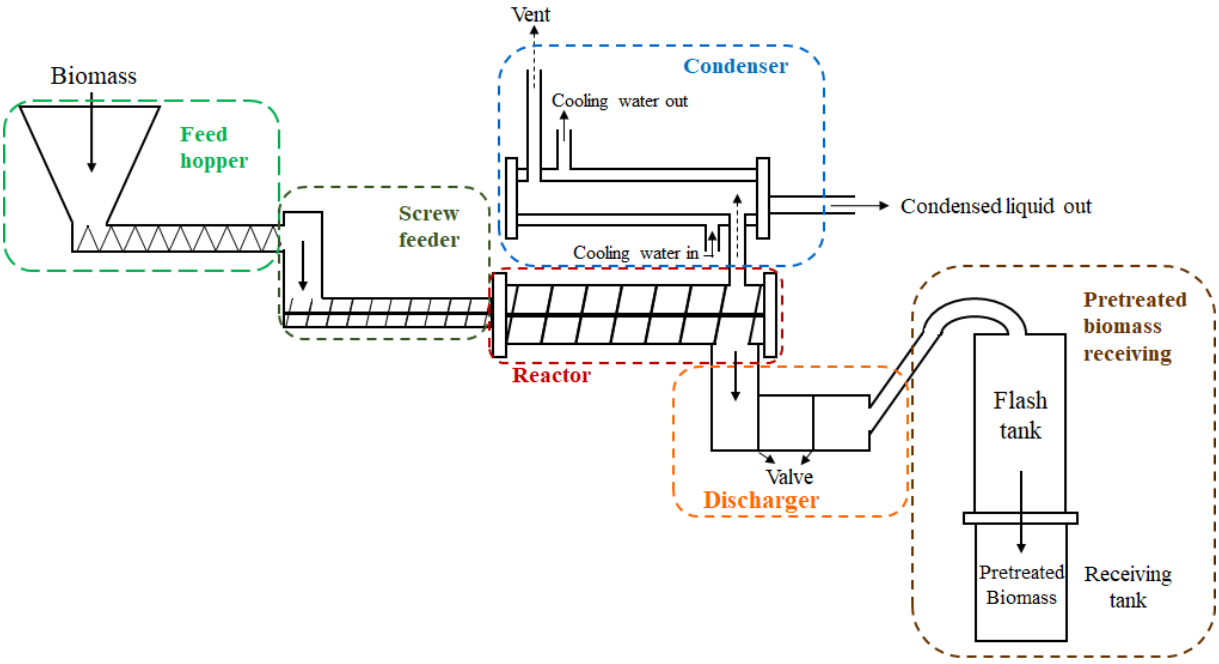
Figure Captions

Fig. 1 Configuration of continuous pretreatment reactor

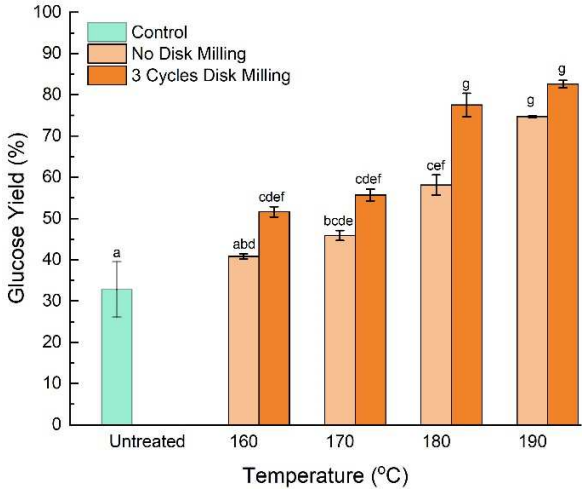
Fig. 2 Sugar yields from pilot-scale continuous hydrothermal pretreatment combined with disk milling process. a: glucose yields; b: xylose yields.

Fig. 3 Sugar yields from lab-scale hot water pretreatment combined with disk milling process. a: glucose yields; b: xylose yields.

Fig. 4 Surface area of raw and pretreated bioenergy sorghum. a: PCH pretreatment combined with disk milling; b: LHW pretreatment combined with disk milling


Vent
Biomass
Condenser
Cooling water out
Feed hopper
Screw feeder
Condensed liquid out
Cooling water in
Pretreated biomass receiving
Reactor
Flash tank
Valve
Discharger
Pretreated Biomass
Receiving tank

a.


Control
No Disk Milling
3 Cycles Disk Milling
Glucose Yield (%)
Temperature (°C)
Untreated
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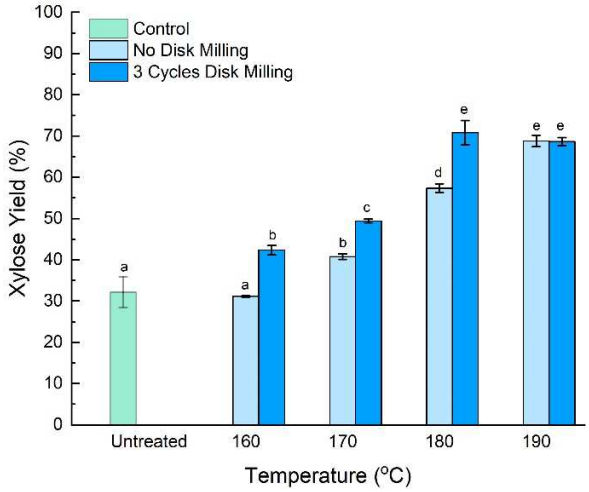
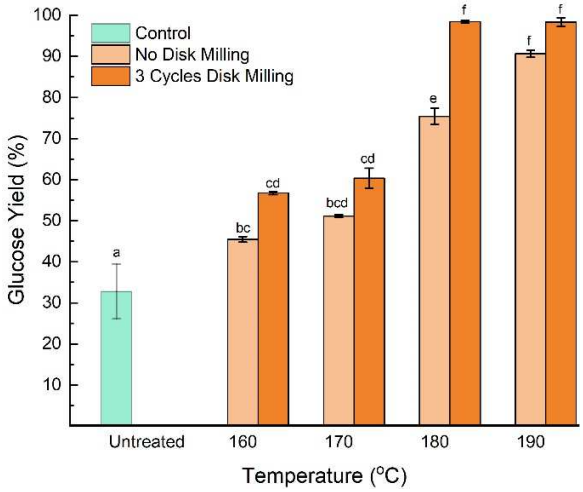
170
180
190
a
abd
cdef
bcde
cdef
cef
g
g
g
g

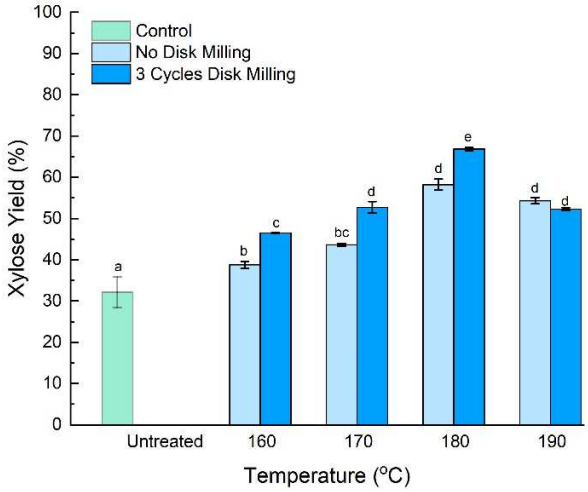
b.


Control
No Disk Milling
3 Cycles Disk Milling
Xylose Yield (%)
Temperature (°C)
Untreated
160
170
180
190
a
a
b
b
c
d
e
e
e

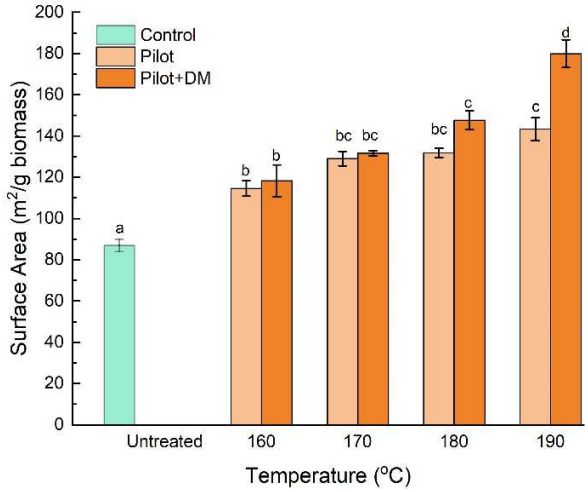
a.


Control
No Disk Milling
3 Cycles Disk Milling
Glucose Yield (%)
100
90
80
70
60
50
40
30
20
10
a
bc
cd
bcd
cd
e
f
f
f
Untreated
160
170
180
190
Temperature (°C)

b.


Control
No Disk Milling
3 Cycles Disk Milling
Xylose Yield (%)
100
90
80
70
60
50
40
30
20
10
0
a
b
c
bc
d
d
d
e
d
d
Untreated
160
170
180
190
Temperature (°C)

a.


Control
Pilot
Pilot+DM
Surface Area (m²/g biomass)
Temperature (°C)
Untreated
160
170
180
190
a
b
b
bc
bc
bc
c
c
d

b.

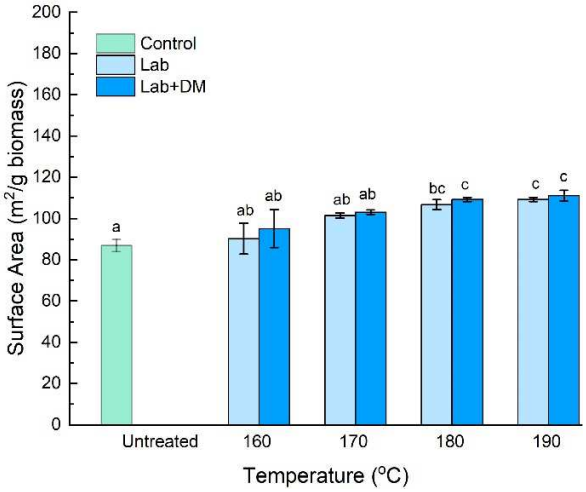

Control
Lab
Lab+DM
Surface Area (m²/g biomass)
Temperature (°C)
Untreated
160
170
180
190
a
ab
ab
ab
ab
bc
c
c
c

Table 1 Compositions (% w/w, db) of raw and hydrothermal pretreated bioenergy sorghum samples

Pretreatment condition	S.F. (Log Ro)[1]	Extractives	Glucan	Xylan	AIL[1]	ASL[1]	Ash
Raw (untreated)	N/A	11.82±0.83	38.77±0.38	21.76±0.09	14.27±0.23	1.88±0.04	2.72±0.10
PCH[1] Pretreated Biomass[2]							
160°C PCH for 10 min	2.77	12.74±0.10	39.66±1.53	19.04±2.34	14.98±1.26	1.58±0.02	4.31±0.34
170°C PCH for 10 min	3.06	14.03±1.03	38.46±1.28	18.50±0.69	14.75±1.04	1.60±0.03	4.39±0.34
180°C PCH for 10 min	3.36	16.01±0.89	39.20±2.64	16.58±3.57	14.78±1.19	1.53±0.14	4.37±0.01
190°C PCH for 10 min	3.65	28.13±0.57	40.72±1.02	10.28±0.54	12.84±0.68	0.9±0.17	4.49±0.08
LHW[1] Pretreated Biomass[2]							
160°C LHW for 10 min	2.77	12.42±0.48	39.91±0.11	21.81±1.21	14.32±0.12	1.72±0.03	2.12±0.45
170°C LHW for 10 min	3.06	14.38±0.74	39.33±2.04	19.16±2.83	15.34±0.61	1.60±0.07	2.40±0.04
180°C LHW for 10 min	3.36	15.69±0.11	39.89±0.26	17.00±0.34	13.90±0.04	1.25±0.08	2.22±0.12
190°C LHW for 10 min	3.65	27.28±0.89	40.14±3.37	7.73±1.08	12.06±0.14	0.95±0.01	1.94±0.12

Results are presented as mean value ± standard deviation

[1]Abbrivations: PCH: pilot-scale continuous hydrothermal pretreatment; LHW: lab-scale hot water pretreatment; S.F.: severity factor; AIL: acid insoluble lignin; Ash: acid soluble lignin

[2]Compositional analysis was performed on whole freeze dried hydrolysate.

Table 2 Inhibitors from lab-scale hot water pretreatment (LHW) (g/L)

Pretreatment condition	S.F. (Log Ro)	Lactic acid	Formic acid	Acetic acid	Levulinic acid	HMF	Furfural
160°C for 10 min	2.77	BDL	BDL	BDL	BDL	BDL	BDL
170°C for 10 min	3.06	BDL	BDL	BDL	BDL	BDL	BDL
180°C for 10 min	3.36	BDL	BDL	BDL	BDL	BDL	BDL
190°C for 10 min	3.65	0.22±0.07	0.50±0.17	1.77±0.10	BDL	BDL	0.46±0.14

Results are presented as mean value ± standard deviation
S.F.: severity factor
HMF: 5-Hydroxymethylfurfural
BDL: below detectable limits of HPLC (0.001% w/w)

Table 3 Inhibitor generations from PCH and LHW pretreatments (g/L)

Pretreatment condition	S.F. (Log Ro)	Lactic acid	Formic acid	Acetic acid	Levulinic acid	HMF[1]	Furfural
Raw (untreated)	N/A	2.260±0.608	0.115±0.007	0.690±0.297	BDL[3]	BDL	BDL
PCH pretreatment							
160°C PCH for 10 min	2.77	BDL	BDL	0.845±0.078	BDL	BDL	BDL
160°C PCH for 10 min+DM[2]	2.77	BDL	BDL	1.025±0.007	BDL	BDL	BDL
170°C PCH for 10 min	3.06	BDL	BDL	1.045±0.049	BDL	BDL	BDL
170°C PCH for 10 min+DM	3.06	BDL	BDL	1.170±0.028	BDL	BDL	BDL
180°C PCH for 10 min	3.36	0.045±0.007	0.085±0.007	1.410±0.014	BDL	BDL	BDL
180°C PCH for 10 min+DM	3.36	0.035±0.007	0.095±0.007	1.440±0.269	BDL	BDL	BDL
190°C PCH for 10 min	3.65	0.085±0.007	0.200±0.003	1.595±0.025	0.037±0.007	BDL	BDL
190°C PCH for 10 min+DM	3.65	0.075±0.006	0.195±0.007	1.530±0.081	0.036±0.007	BDL	BDL
LHW pretreatment							
160°C LHW for 10 min	2.77	0.075±0.007	BDL	1.195±0.191	BDL	BDL	BDL
160°C LHW for 10 min+DM	2.77	0.075±0.007	BDL	1.615±0.021	BDL	BDL	BDL
170°C LHW for 10 min	3.06	0.075±0.008	BDL	1.475±0.007	BDL	BDL	BDL
170°C LHW for 10 min+DM	3.06	0.075±0.006	BDL	1.600±0.099	BDL	BDL	BDL
180°C LHW for 10 min	3.36	0.085±0.007	BDL	1.920±0.127	BDL	BDL	BDL
180°C LHW for 10 min+DM	3.36	0.075±0.008	BDL	2.115±0.106	BDL	BDL	BDL
190°C LHW for 10 min	3.65	0.105±0.006	0.380±0.028	2.245±0.007	BDL	BDL	BDL
190°C LHW for 10 min+DM	3.65	0.095±0.021	0.365±0.049	2.244±0.014	BDL	BDL	BDL

Results are presented as mean value ± standard deviation

[1]HMF: 5-Hydroxymethylfurfural

[2]DM: Three disk milling cycles

[3]BDL: below detectable limit of HPLC (0.001%, w/w)